REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

Public reporting burden for this collection of informati- gathering and maintaining the data needed, and compl collection of information, including suggestions for red Davis Highway, Suite 1204, Arlington, VA 22202-4302.	on is estimated to average 1 hour leting and reviewing the collection lucing this burden, to Washington and to the Office of Management a	oer response, including the time for re of information. Send comments rega Headquarters Services, Directorate fo and Budget, Paperwork Reduction Pro	eviewing instructions, searching existing data sources, irding this burden estimate or any other aspect of this r Information Operations and Reports, 1215 Jefferson ject (0704-0188), Washington, DC 20503.
1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE 5/26/99	3. REPORT TYPE AN Final Repo	D DATES COVERED ort 9/1/93 - 8/31/96
4. TITLE AND SUBTITLE Cloning of P8, A Transcription Factor Required of Skeletogenic Gene Expression			5. FUNDING NUMBERS
6. AUTHOR(S) PI: Eric H. Davidson			N00014-93-1-1400
7. PERFORMING ORGANIZATION NAME(S Division of Biology California Institute of T 1200 E. California Blvd. Pasadena, CA 91125	echnology		8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY Office of Naval Resea 800 N, Quincy St. Arlington, VA 22217-	arch	_	10. SPONSORING / MONITORING AGENCY REPORT NUMBER
11. SUPPLEMENTARY NOTES		19990	601 082
Distribution Unlimite			126. DISTRIBUTION CODE
13. ABSTRACT (Maximum 200 words)	kat kapatus valuutuuri take tiin katuuri valutus katulusta katuuri katuuri valuuta katuuri katuuri katuuri val Katuuri	ra inandrandrandrandrandrandrandrandrandrand	Patter Particular Appendix accorded a principa de constante de mandata de mandata de superior de reducirio per de constante de constant
now as Sp(G/C)F-1) is genes, and probably a was: <i>i</i> , purified by af factor was sequenced; bacteria and an antibod aspect of its structure wembryo nuclear extractions are also presented cDNA. We shoed that mRNA, all used more sequence reveals Sp(G,	nteracts with reg cts as a general en finity chromatogr iv, its DNA bindin by generated; vi, it was analyzed. The tin five different ed in cell-free trans t—unusually—the cor less equally, (C)F-1 to be a nove to the egg in relative	ulatory regions of nhancer protein. Deaphy; ii, microsequing site was determined by provenance was defermed atterning that the factorial forms of decreasing slation using synthem are five successed thus accounting for DNA-binding proceed and protein that the factorial synthem are five successed.	ed (formerly known as P8, many sea urchin embryo buring this year this factor ence was obtained; iii, the ned; v, it was expressed in etermined; and an unusual actor appears in sea urchin molecular weight. These tic mRNA from the cloned ive ATG start sites in this or five nested forms. The tein, unlike any in the data during oogenesis and then

14. SUBJECT TERMS 15. NUMBER OF PAGES sea urchin, transcription factor, embryo 16. PRICE CODE 17. SECURITY CLASSIFICATION OF REPORT SECURITY CLASSIFICATION OF THIS PAGE SECURITY CLASSIFICATION OF ABSTRACT 20. LIMITATION OF ABSTRACT Unclassified Unclassified Unclassified UL

FINAL REPORT

Grant #: N00014-93-1-1400

PRINCIPAL INVESTIGATOR: Eric H. Davidson

INSTITUTION: California Institute of Technology

GRANT TITLE: Cloning of P8, A Transcription Factor Required for Skeletogenic

Gene Expression

AWARD PERIOD: 9/1/93 - 8/31/96

<u>OBJECTIVE:</u> This is an AASERT Graduate Training Project, the object of which is to isolate, purify, clone, and characterize transcription factors that interact with the regulatory domains of skeletogenic genes in the sea urchin.

<u>APPROACH</u>: The known target site of the factor Sp(G/C)F-1, which interacts with the SM50 and SM30 skeletogenic genes, provides the means to purify this protein, clone it and characterize its interaction with DNA, its own structure, its provenance, and its particular function.

ACCOMPLISHMENTS: The transcription factor toward which this project was directed (formerly known as P8, now as Sp(G/C)F-1) interacts with regulatory regions of many sea urchin embryo genes, and probably acts as a general enhancer protein. During this year this factor was: *i*, purified by affinity chromatography; *ii*, microsequence was obtained; *iii*, the factor was sequenced; *iv*, its DNA binding site was determined; *v*, it was expressed in bacteria and an antibody generated; *vi*, its provenance was determined; and an unusual aspect of its structure was analyzed. The latter is that the factor appears in sea urchin embryo nuclear extract in five different forms of decreasing molecular weight. These forms are also presented in cell-free translation using synthetic mRNA from the cloned cDNA. We shoed that—unusually—there are five successive ATG start sites in this mRNA, all used more or less equally, thus accounting for five nested forms. The sequence reveals Sp(G/C)F-1 to be a novel DNA-binding protein, unlike any in the data banks. It is loaded into the egg in relatively large quantities during oogenesis and then appears in embryo nuclei.

<u>SIGNIFICANCE</u>: In the course of this project the Graduate Fellow carrying out the work acquired professional expertise in the complete range of molecular technologies required for transcription factor isolation and characterization. As a result we now have our hands on another of the regulatory molecules that control gene expression, including skeletogenic gene expression.

PATENT INFORMATION: None

AWARD INFORMATION: Received Ph.D.

PUBLICATIONS:

- Zeller, R. W., Coffman, J. A., Harrington, M. G., Britten, R. J. and Davidson, E. H. SpGCF1, a sea urchin embryo transcription factor, exists as five nested variants encoded by a single mRNA. *Dev. Biol.* **169**, 713-727, 1995.
- Zeller, R. W., Britten, R. J. and Davidson, E. H. Developmental utilization of SpP3A1 and SpP3A2: Two proteins which recognize the same DNA target site in several sea urchin gene regulatory regions. *Dev. Biol.* **170**, 75-82, 1995.
- Zeller, R. W., Griffith, J. D., Moore, J. G., Kirchhamer, C. V., Britten, R. J. and Davidson, E. H. A multimerizing transcription factor of sea urchin embryos capable of looping DNA. *Proc. Natl. Acad. Sci. USA* **92**, 2989-2993, 1995.